TANNIC ACID MIMICKING DENDRIMERS AS A STABILIZING NANOMORDANT IN VASCULAR TISSUE ENGINEERING

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Abstract: Chemical stabilization resulting in increased resistance to proteolytic degradation is one of the approaches in prevention of post-implantational aneurysm development scaffolds. Recently, tannic acid (TA) and tannic acid mimicking dendrimers (TAMD) have been suggested as potential stabilization agents for collagen and elastin. The aim of this work was to determine the stabilizing effects of TAMD on decellularized natural scaffolds. Vascular scaffolds fabricated from small intestine submucosa (SIS) and SIS plane sheets (Cook Biotech Inc.) were used. The biomechanical properties of the SIS vascular graft segments treated with TA and TAMD were tested. The effect of TAMD treatment on resistance to proteolytic degradation was evaluated by measuring biomechanical properties of TAMD stabilized and non-stabilized SIS specimens after incubation in collagenase solution. It was shown that treatment with TA as well as with TAMD increased the strength of tubular SIS as well as their resistance to proteolytic biodegradation manifested by preservation of biomechanical properties after collagenase treatment. Transmission electron microscopy demonstrated that treatment with TAMD increased the periodical pattern typical of collagen fiber ultrastructure as a result of the "mordant" effect. The treatment with TAMD induced a small, but detectable cross-linking effect, thus rendering SIS scaffolds more resistant to proteolytic degradation.

Introduction

The small diameter vascular graft is the “holy grail” of cardiovascular surgery. However, all existing synthetic vascular grafts have failed as coronary bypasses due to early thrombosis and late anastomotic intimal thickening. It is generally believed that a tissue engineering approach could solve this problem by creating a functional living small diameter vascular graft. The emerging field of vascular tissue engineering has made impressive progress during the last decade [1-3. However, ideal synthetic biodegradable scaffolds are still not commercially available. Furthermore, there are not even non-biodegradable synthetic scaffolds that exactly mimic the non-linear biomechanical behavior of natural arteries. In this situation the tissue engineered vascular graft fabricated from decellularized natural scaffolds represents a realistic alternative. As shown in previously reported animal studies, decellularized natural scaffolds implanted as vascular grafts could be patent up to 6 years in dogs [4]. The technologies for decellularization of naturally derived scaffolds are already well developed and are constantly being improved and optimized [5]. It was recently shown that bone marrow derived adult stem cells seeded on vascular natural scaffolds can differentiate both into endothelial and smooth muscle cell lineages [6], suggesting that effective re-cellularization of decellularized scaffolds is feasible. This suggests that at least early thrombosis may not be a major problem anymore. However, prevention of aneurysm development is still unsolved and represents a great challenge for tissue engineers.

One possible way to increase the resistance of natural scaffolds to proteolytic degradation is to use chemical stabilizing and/or cross-linking agents. Recently it was shown that there is competition between cross-linking and vascular remodeling. By fully cross-linking a natural vascular scaffold, the capacity for remodeling and recellularization is reduced, whereas fresh unstabilized decellularized vascular scaffolds undergo fast proteolytic degradation and thus could be prone to aneurysm development [7]. Thus, in order to be successful, acellular vascular scaffolds must be both biomechanically compliant and resistant to proteolytic degradation in order to prevent a possible aneurysm development after implantation.

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Recently tannic acid (TA) and tannic acid mimicking dendrimers (TAMD) have been suggested as potential cross-linking agents for collagen [8,9]. It was also shown that addition of TA to glutaraldehyde (Glut) pretreatment dramatically improves elastin and collagen stabilization in cardiovascular implants as evidenced by an increased resistance to proteolytic degradation. Moreover, TA has 10 times lower toxicity as compared to Glut [10]. The main advantage of using TAMD is that they are more stable (due to their chemical structure) than TA, thus they do not generate hepatotoxic products of TA biodegradation such as free gallic acid [9].

The aim of this work is to determine the stabilizing effects of TAMD on decellularized natural tubes made of small intestinal submucosa (SIS), a promising material for vascular scaffolds [11]. We hypothesize that it is possible to find an optimal concentration of TAMD which will improve scaffold resistance to proteolytic degradation. Here, we are reporting the detectable moderate effect of TA and TAMD treatment on biomechanical properties of SIS, and for the first time are providing evidence that treatment with TAMD stabilizing agent significantly improves resistance of SIS scaffolds to proteolytic degradation.

Materials and Methods

2.1. Material and reactive

To investigate the influence of different cross-linking agents, small intestinal submucosa (SIS) vascular grafts (diameter 7 mm, length 5 cm) (Cook Biotech Inc., West Lafayette, IN) [11] and SIS plane sheets were used as experimental material. The wall thickness of the vascular grafts was 0.132 ± 0.014 mm. The initial wall thickness h_0 was measured with an electronic digital caliper (Starrett) to ± 0.001 mm accuracy. Tannic acid was purchased from Sigma (St. Louis, MO). Tannic acid mimicking dendrimers were synthesized as described before [9]. Glutaraldehyde was obtained from Polysciences, Inc. (Warrington, PA).

2.2. Methods of SIS treatment with TA and TAMD

All tubular specimens were treated with different solutions dissolved or diluted in media M199 and were divided into five groups (n = 7 per group):

Vascular grafts treated with 0.5% Glut for 24 h;
Vascular grafts treated with 2% tannic acid mimicking dendrimers (D1 - Mol. Wt.: 544.46) for 24 h.
Vascular grafts treated with 0.2% tannic acid mimicking dendrimers (D1 - Mol. Wt.: 544.46) for 24 h;
Vascular grafts treated with 2% tannic acid for 24 h;
Vascular grafts treated only with media M199 for 24 h;

To investigate the cross-linking effect of different sizes (molecular weights) of dendrimers [9], SIS sheets were used and divided into four treatment groups (n = 5 per group):

Specimens treated only with media M199 for 24 h (wall thickness = 0.198 ± 0.013 mm);
Specimens treated with 0.2 % tannic acid mimicking dendrimers (D1 - Mol. Wt.: 544.46) for 24 h (wall thickness = 0.220 ± 0.050 mm);
Specimens treated with 0.2 % tannic acid mimicking dendrimers (D2 - Mol. Wt.: 1293.15) for 24 h (wall thickness = 0.188 ± 0.051 mm);
Specimens treated with 0.2 % tannic acid mimicking dendrimers (D3 - Mol. Wt.: 2790.53) for 24 h (wall thickness = 0.195 ± 0.058 mm).

The treatment of all samples occurred in an incubator at 37º C.

2.*3. Collagenase treatment*

To investigate enzymatic degradation, half of all samples were incubated in 100 Uml^{-1} collagenase (10 U per 1 g of SIS) solution for 2 hours. Tensile tests were performed to estimate changes in mechanical properties of the material after degradation.

2.4. Biomechanical testing by internal pressure

The biomechanical properties of the vascular scaffolds manufactured from SIS were investigated using a perfusion bioreactor [12], which included a biomechanical control system. This system consisted of two pressure transducers (Entran, EPB-CO2-5P-/RQ) and a digital TV camera (Kodak, MDS 100) connected to the computer. The pressure inside the tubular construct is calculated as a mean value of the two transducers. Diameter changes of the vascular graft were sensed optically with the TV camera, which was coupled with a suitable lighting system for high contrast. This system allows the recording of a relationship between the diameter of the vascular graft and inner pressure.

In these experiments, SIS vascular graft segments were gradually loaded by increasing the internal pressure from 0 to 160 mm Hg at the constant values of longitudinal stretch ratio λ_1 = 1.0. The pressure was elevated in increments of 10 mm Hg and was maintained for 1 min after which the measurements were recorded. The thickness of the wall during the experiment was calculated using the condition of incompressibility of the material: $h = h_0 * \lambda_3$, where h_0 is the initial thickness of the vascular graft wall.

$\lambda_3 = 1/(\lambda_1 \times \lambda_2)$ - the stretch ratio in the radial direction, where $\lambda_2 = D/D_0$ - stretch ratio in the circumferential direction. D_0 is the initial diameter of the specimen, and D is the observed length and diameter of the vascular graft during loading.

In this case $h = h_0 (D_0 / D)$. The mean stress in the circumferential (σ_2) direction was computed as:

$$\sigma_2 = (pR) / h, \qquad (1)$$

where h and R are the thickness and external radius of the vascular graft during pressurizing, respectively, and p is the intraluminal pressure.

The circumferential strain (ε_2) of the vascular graft is calculated as:

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$$\varepsilon_2 = (D - D_0)/D_0, \qquad (2)$$

where D_0 is the initial (non-deformed) diameter of the vascular graft and D is the diameter after deformation.

2.5. Tensile tests

Dumb–bell shaped (5.0 mm wide in the middle, length 5 cm) samples (n = 110) of SIS vascular grafts in longitudinal direction and sheets were cut with a template in order to perform tensile tests. These studies investigated the effect of the different treatments, as well as the influence of collagenase digestion (described above). Tensile tests were performed using an MTS tensile test system until rapture. (The 858 Mini Bionix II Test System). All specimens were wet and during experiment the specimens were kept continuously moist with room temperature. Displacement was measured automatically between grips. Force-elongation curves were recorded at an elongation rate of 5mm min^{-1}, and then ultimate stress and ultimate strain were calculated. The incremental modulus of elasticity was calculated also using MTS system soft at the ultimate stress (one point was chosen as ultimate stress and another point - the stress which was lower then the ultimate stress on 10%).

2.6. Transmission electron microscopy

TAMD-treated and non-treated specimens were fixed in 2.5% of buffered Glut solution based on medium 199, followed by post-fixation in 1.5% osmium tetroxide before dehydration and sequential embedding in Epon 812 resin (Polyscience Inc). Sectioning was performed with a diamond knife on an LKB Ultratome IV microtome and sections were collected on copper grids. Different staining protocols using TAMD as the mordant agent were followed by staining with uranyl acetate. A nanomordant could be defined as a nanosize (0.1nm -100nm) natural or synthetic chemical (or compound) that combines with both specific cellular or extracellular tissue components and the staining substance, thereby facilitating a staining reaction that otherwise would not occur [20]. Ultra-thin sections were investigated using a JEOL 1210 transmission electron microscope , operated at 60 kV.

2.*7. Differential scanning calorimetry*

The thermal denaturation temperature (T_d), a common indicator of collagen crosslinking density [21], was measured in SIS sheet samples from each treatment group (n = 3) using a differential scanning calorimeter (DSC) (Perkin-Elmer DSC 7; Boston, MA). Tissue samples (approximately 3 mm x 3 mm) were sealed in aluminum pans, heated at a rate of 10°C/min from 20°C to 100°C and T_d determined as the temperature measured at the endothermic peak.

2.8. Statistics

Groups of data were analyzed by single-factor ANOVA. For pair-wise comparisons, heteroscedastic *t* – tests were used to determine significance. A *p* value less than 0.05 was considered statistically significant.

Results

3.1. Mechanical properties of SIS grafts

The stress-strain relationship for all SIS graft specimens was non-linear (Figure 1), except for vascular grafts treated with 0.5% Glut. Vascular grafts incubated in 0.5% Glut solution were more rigid than other treatment groups and had a linear stress-strain relationship. It was shown that TAMD provides more effective cross-linking than TA, and results in a more compliant vascular tube than using 0.5% Glut solution. Circumferential strain at a circumferential stress of 700 kPa for samples treated with 0.5% GA was 0.024 ± 0.008, while it was 0.041 ± 0.009 for 2% TAMD and 0.052 ± 0.010 for 0.2% TAMD (Figure 1).

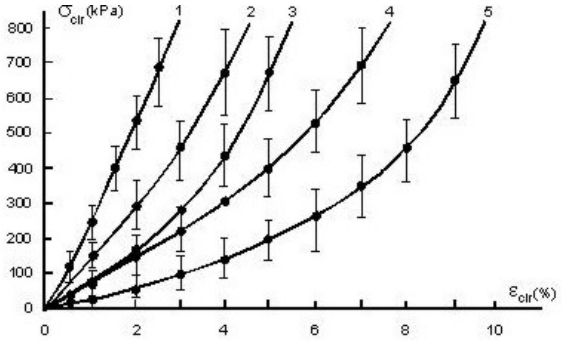

Figure 1: Stress – strain relationship in a circumferential direction for SIS vascular grafts treated with different solutions for 24 hours: 1 - 0.5 % glutaraldehyde solution; 2 - 2 % tannic acid mimic dendrimer solution; 3 - 0.2 % tannic acid mimic dendrimer solution; 4 - 2% tannic acid solution and 5 - media M199.

The ultimate stress of the samples treated with 2% TAMD was greater than those treated with 2 % TA and 0.5 % Glut (81.0 ± 13.3 MPa, 69.9 ± 9.8 MPa and 59.3 ± 11.4 MPa, respectively) (Figure 2).

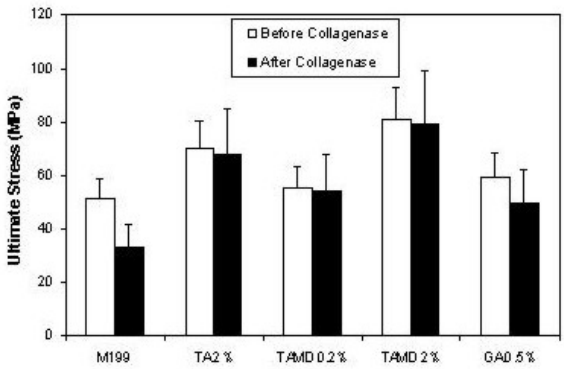

Figure 2: Ultimate stress in circumferential direction for SIS vascular grafts treated with media M199, 2% tannic acid, 2% tannic acid mimic dendrimers, 0.2% tannic acid mimic dendrimers and 0.5% glutaraldehyde for 24 hours, before and after their incubation with 100 Uml^{-1} collagenase for 2 h.

The ultimate stress of samples treated with 0.2 % TAMD solution was not different (p > 0.5) from the

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0.5% Glut group. Before and after collagenase treatment, these values were not significantly different from each other for specimens treated with 0.2 % TAMD and 2% TAMD solutions ($p>0.05$). Statistical differences ($p<0.05$) existed for the ultimate stress of the samples treated with 0.5 % Glut solution before and after collagenase (59.2 ± 8.6 MPa and 49.7 ± 10.2 MPa, respectively) and for samples treated only by media M199 before and after collagenase (51.2 ± 8.1 MPa and 33.3 ± 6.8 MPa, respectively).

Incremental modulus of elasticity of the samples treated with 2% TA and 2% TAMD solutions were statistically different ($p<0.05$) from one another (Figure 3), 255.9 ± 43.3 and 380.5 ± 62.6 MPa, respectively.

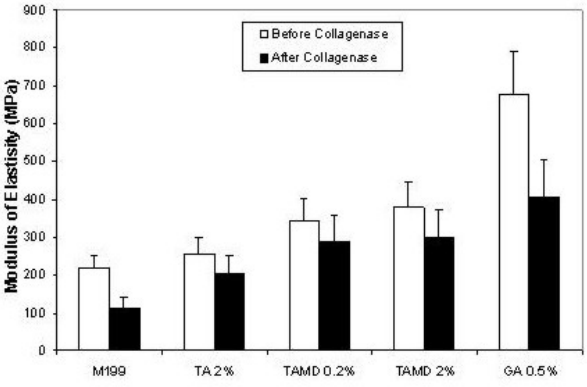

Figure 3: Incremental modulus of elasticity in the longitudinal direction at the ultimate stress for SIS vascular grafts treated with media M199, 2% tannic acid, 2% tannic acid mimic dendrimers, 0.2% tannic acid mimic dendrimers and 0.5% glutaraldehyde for 24 hours, before and after their incubation in the 100 Uml^{-1} collagenase solution for 2 h.

However, there was no difference between modulus of elasticity for samples treated with 0.2% TAMD and 2% TAMD solutions ($p>0.5$). The highest modulus of elasticity was observed in samples treated with 0.5% Glut solution, while the lowest was found in samples treated only with media M199 (677.2 ± 114.8 MPa and 217.5 ± 38.4 MPa, respectively).Decreases in modulus of elasticity were most notable in samples treated with media M199 and 0.5% Glut solution (48.9 ± 12.2 % and 39.2 ± 9.6 MPa, respectively). Statistical differences were not found for percent changes in modulus of elasticity for samples treated with 2% TA, 2% TAMD, and 0.2% TAMD solutions ($p>0.05$). Furthermore, these values are significantly lower ($p<0.5$) than for samples treated with media M199 and 0.5% Glut solution.

3.2. Mechanical properties of SIS sheets treated with differentTA dendrimers

Investigation of the biomechanical properties of SIS sheet samples (Figure 4) shows that there is a statistical difference ($p<0.05$) between ultimate stress for samples treated with D1 as compared to media M199 alone or D2 dendrimer treatments (17.54 ± 1.27 MPa, 15.23 ± 0.91 MPa and 15.73 ± 3.16 MPa, respectively). Despite the fact that the average of the ultimate stress of specimens treated with dendrimer D1 is greater than those treated by D2 and D3, there is no statistical difference ($p>0.1$).

After enzymatic degradation in 100 Uml^{-1} collagenase for 2 hours, the ultimate stress of samples treated with media M199 and D3 essentially decreased ($p<0.05$) in comparison to samples treated with D1 and D2 (9.20 ± 0.73 MPa, 7.56 ± 2.51 MPa and 12.68 ± 2.44 MPa, 12.47 ± 2.51 MPa, respectively after collagenase).

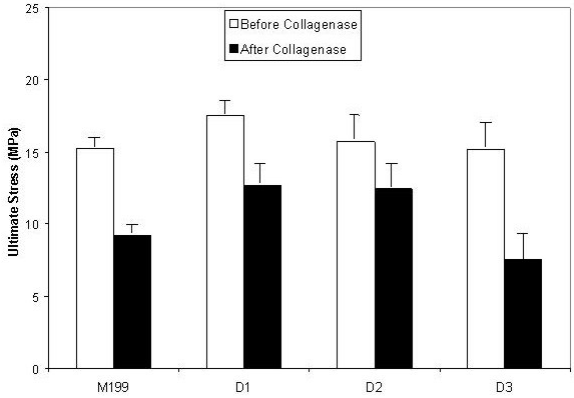

Figure 4: Ultimate stress for SIS sheets treated with media and 0.2% solutions of different dendrimers before and after collagenase. M199 – media, D1-dendrimer with Mol. Wt.: 544.46, D2 – dendrimer with Mol. Wt.: 1293.15 and D3 – dendrimer with Mol. Wt.: 2790.53 (these are described in methods already).

There is no statistical difference between ultimate stress of samples treated with D1 and D2 ($p>0.05$), both before enzymatic degradation (17.54 ± 1.27 MPa and 15.73 ± 3.16 MPa, respectively), and after degradation (12.68 ± 2.44 MPa and 12.47 ± 2.51 MPa, respectively).

3.3. Transmission electron microscopic analysis

Electron microscopic investigation demonstrated that treatment with TAMD significantly increased a periodical pattern typical for a collagen fiber ultrastructure as a result of "mordant" effect. The individual striated fibers appear as electron lucid ribbons in sections that were left unstained or treated only with osmium tetroxide. Sections of SIS specimens that were mordanted with TAMD appeared much the same as if no mordanting had been performed, except for a better visibility of the periodic striations. When SIS specimens mordanted with TAMD were followed by fixation with osmium tetroxide, the collagen fibers appeared as electron dense ribbons with clearly visible striations.

3.4. Differential scanning calorimetry (DSC) analysis

DSC (Figure 5) revealed that most TAMD preparations induced a small, but significant increase in thermal denaturation temperature (T_d), indicative of formation of a limited number of crosslinks between TAMD and matrix components in SIS. While D1 values were not statistically different from M199 media controls ($p>0.1$), the D2 and D3 preparations exhibited higher T_d ($p<0.05$) as compared to controls. D1 values

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were not statistically different from D3 ($p>0.1$). The most significant increase in T_d values were those for the intermediary molecular weight TAMD, namely D2, possibly indicating that there is an optimal molecular weight requirement for TAMD to stabilize collagen. As expected, glutaraldehyde fixation increased T_d to more than 87 ^oC.

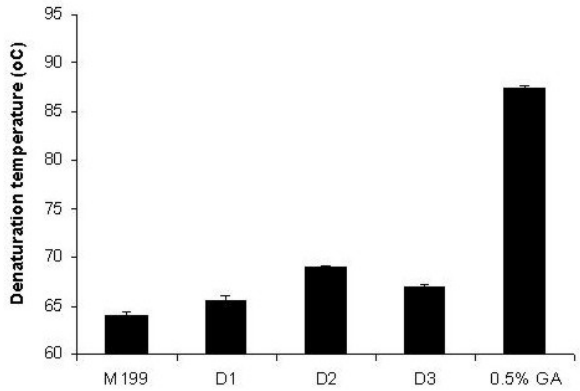

Figure 5: Thermal denaturation temperatures of SIS sheets treated with media and 0.2% solutions of different dendrimers, as acquired through differential scanning calorimetry (DSC). M199 – media, D1 – dendrimer with Mol. Wt.: 544.46, D2 – dendrimer with Mol. Wt.: 1293.15 and D3 – dendrimer with Mol. Wt.: 2790.53 (these are described in methods already).

Discussion

Decellularized vascular scaffolds were proposed to be used as vascular grafts two decades ago [11]. Optimization of decellularization technologies has allowed 94% patency up to 6 years after implantation of acellular scaffolds in dogs [4]. However, the risk of aneurysm development precluded the clinical use of decellularized vascular scaffolds. The rationale behind this research was to use non-toxic TAMD as a novel nanomordant for stabilization of acellular natural scaffolds designed to be used as vascular grafts.

There are several approaches to stabilizing the ECM by using different cross-linking agents [5]. In the search for ECM stabilizing agents, natural plant-derived phenolic substances such as genipin have become increasingly popular due to their low toxicity [7]. Recently it was shown that TA could be used as a stabilizer for collagen [8] and elastin [10]. However, in burn patients hepatotoxic effects following topical application of TA were reported [8, 9]. Although TA is 10 times less toxic than glutaraldehyde, a common cross-linking agent (LD50 of TA orally in mice=6 g/kg vs. 0.6 g/kg for Glut, as per Merck Index), possible elimination of its potential hepatotoxic effect is still a desirable goal.

Recently, structurally related analogues of TA that combine a higher stability (thus reducing hepatoxicity) with a similar biological activity have been synthesized. To achieve this objective, Halkes et al. [9] have chosen to synthesize first-, second-, and third-generation dendrimers containing two, four, and eight galloyl moieties, respectively. Dendrimers were used with a 3,5-di(2-aminoethoxy)benzoic acid repeating unit. A convergent synthesis was used that started with the benzyl protection of the three hydroxyl groups of the starting material methyl gallate. Stability, antioxidant activity, and collagen cross-linking activity of the natural product and its dendrimer analogues were compared. The experimental results on cross-linking of purified skin collagen matrices indicate that polygalloyl dendrimers might be used as new lead compounds in collagen stabilization.

Our data demonstrate that the molecular size of TAMD has a small, but detectable effect on cross-linking activity of TAMD. This suggests that TAMD does not increase the complexity of matrix structure as it occurs in the case of Glut treatment. Moreover, TAMD stabilizing effects are probably based on a different molecular mechanism or have only limited cross-linking activity detectable by the methods employed here. It is important to mention that TEM studies reported in this paper demonstrate the direct binding of TAMD with collagen molecules, resulting in the so-called "mordant effect" [13], as suggested by the increased periodic structure pattern in collagen fibers. The mechanism of stabilization with Glut treatment involves extensive cross-linking and polymer formation within the native SIS matrix, leading to a rise in matrix complexity and thermal stability. In contrast, TAMD treatment may be inducing cross-link formation within existing matrix components, resulting in a material with little added matrix complexity, which is not detectable by current cross-linking methods. Dendrimers D1 and D2 provide a similar degree of collagen cross-linking. However, dendrimer D3 creates weaker bonds between collagen fibers, resulting in a marked decrease in ultimate stress after enzymatic degradation. The reported decline in cross-linking capacity going from D2 to D3 types of TAMD could be speculatively explained by size of D2 TAMD ideally suited to infiltrate in the gap between tropocollagen molecules that compose the collagen fibrils. However, this interpretation needs specially designed systematic studies involving chemical methods.

Although TAMD treatment resulted in a moderate increase in SIS stiffness, these changes are less than those induced by glutaraldehyde treatment. Thus, at least theoretically, it is possible to speculate that TAMD treated acellular vascular grafts would not be subject to post-implantational anastomotic intimal thickening due to mechanical miscompliance.

TA and TAMD can also potentially reduce immunogenicity and have certain antibacterial effects [10]. Moreover, there are some indications that certain components or products of degradation of TA can inhibit collagen proteolysis by interfering with production of matrix metalloproteinases (MMP) via

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inhibiting transcription of the promoter for MMP [14]. However, the main benefit of the proposed TAMD procedure is increasing the resistance of acellular vascular grafts to proteolytic biodegradation with elimination or reduced risk of aneurysm development. In this study, we used biomechanical testing (tensile testing rupture) before and after TAMD treatment in order to estimate the effect of TAMD on resistance to proteolytic biodegradtion. It was shown that in the case of fresh untreated SIS specimens, modulus of elasticity was reduced 50% after exposure to collagenase treatment, whereas in the case of TA, TAMD and Glut, the modulus of elasticity was reduced only by 20-25%. These data support previous investigations of the effect of tannic acid and tannic acid mimicking dendrimers on other collagen and elastin matrices [8-10]. Thus, TAMD significantly increases resistance of SIS specimens to proteolytic degradation.

Although cross-linking and/or stabilization is considered an important step in the preparation of acellular matrix material for cardiovascular implants [5], there is still no consensus in this field. According to one strategy, cross-linking and stabilization is an unnecessary procedure which will only reduce capacity of the implant to remodel. Another strategy reasonably assumes that an optimal cross-linking procedure will improve implant durability and resistance to proteolytic degradation, thus improving long-term performance of cardiovascular implants such as vascular grafts and biological heart valves [5]. This strategy in the development of acellular vascular grafts could be based on complete or maximal elimination of possible post-implantational remodeling and cell invasion. Finally, the third point of view is based on the assumption that ideal implanted acellular scaffolds must have optimal resistance to post-implantational biodegradation, which will allow remodeling or gradual replacement of implanted ECM [7]. The determination of an optimal level of stabilization and cross-linking of natural scaffolds, which will allow cell invasion and proteolytic degradation and safe gradual post-implantational replacement of scaffolds with neosynthesed ECM, must be the subject of further in vivo experiments. However, it is obvious that optimal TA and TAMD treatment could be potentially beneficial and may improve performance of acellular cardiovascular implants or at least provide some initial post-implantational safety .

Conclusions

TAMD treatment of SIS scaffolds provided more stable mechanical properties than Glut and significantly enhanced resistance to proteolytic degradation and might be used as a novel stabilizing nanomordant of decellularized natural vascular scaffolds.

References

[1] L'HEUREUX, N., PAQUET, S., LABBE, R., GERMAIN, L., AND AUGER, F.A. (1998): 'A completely biological tissue-engineered human blood vessel', *Faseb. J.*, **12**, pp. 47-56.

[2] NIKLASON, L.E., GAO, J., ABBOTT, W.M., HIRSCHI, K.K., HOUSER, S., MARINI, R., AND LANGER, R. (1999): 'Functional arteries grown in vitro', *Science*, **284**, pp. 489-493.

[3] NEREM, R.M., SELIKTAR, D. (2001): 'Vascular tissue engineering', *Annu. Rev. Biomed. Eng.*, **3**, pp. 225-243.

[4] WILSON, G.J., COURTMAN, D.W., KLEMENT, P., LEE, J.M., AND YEGER, H. (1995): 'Acellular matrix: a biomaterials approach for coronary artery bypass and heart valve replacement', *Ann. Thorac. Surg.*, **60**, pp. 353-358.

[5] SCHMIDT, C.E., BAIER, J.M. (2000): 'Acellular vascular tissues: natural biomaterials for tissue repair and tissue engineering', *Biomaterials*, **21**, pp. 2215-2231.

[6] KAUSHAL, S., AMIEL, G.E., GULESERIAN, K.J., SHAPIRA, O.M., PERRY T., SUHTERLAND, F.W., RABKIN, E., MORAN, A.M., AND SCHOEN, F.J. (2001): 'Functional small-diameter neovessels created using endothelial progenitor cells expanded ex vivo', *Nat. Med.*, **7**, pp. 1035-1040.

[7] LIANG, H.C., CHANG, Y., HSU, C.K., LEE, M.H., AND SUNG, H.W. (2004): 'Effects of crosslinking degree of an acellular biological tissue on its tissue regeneration pattern', *Biomaterials*, **25**, pp. 3541-3552.

[8] HEIJMEN, F.H., DU PONT, J.S., MIDDELKOOP, E., KREIS, R.W., AND HOEKSTRA, M.J. (1997): 'Cross-linking of dermal sheep collagen with tannic acid', *Biomaterials*, **18**, pp. 749-754.

[9] HALKES, S.B., VRASIDAS, I., ROOIJER, G.R., VAN DEN BERG, A.J., LISKAMP, R.M., AND PIETERS R.J. (2002): 'Synthesis and biological activity of polygalloyl-dendrimers as stable tannic acid mimics', *Bioorg. Med. Chem. Lett.*, **12**, pp. 1567-1570.

[10] ISENBURG, J.C., SIMIONESKU, D.T., AND VYAVAHARE, N.R. (2004): 'Elastin stabilization in cardiovascular implants: improved resistance to enzymatic degradation by treatment with tannic acid', *Biomaterials*, **25**, pp. 3293-3302.

[11] HODDE, J. (2002): 'Naturally occurring scaffolds for soft tissue repair and regeneration', *Tissue Eng.*, **8**, pp. 295-308.

[12] MIRONOV, V., KASYANOV, V., MCALLISTER, K., OLIVER, S., SISTINO, J., AND MARKWALD, R. (2003): 'Perfusion bioreactor for vascular tissue engineering with capacities for longitudinal stretch', *J. Craniofac. Surg.*, **14**, pp. 340-347.

[13] AFZELIUS, B.A. (1992): 'Section staining for electron microscopy using tannic acid as a mordant: a simple method for visualization of glycogen and collagen', *Microsc. Res. Tech.*, **21**, pp. 65-72.

[14] ATA, N., OKU, T., HATTORI, M., FUJII, H., NAKAJIMA, M., AND SAIKI, I. (1996): 'Inhibition by galloylglucose (GG6-10) of tumor invasion

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through extracellular matrix and gelatinase-mediated degradation of type IV collagens by metastatic tumor cells', *Oncol. Res*., **8**, pp. 503-511.

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